International Journal of Environment, Agriculture and Biotechnology
Vol-9, Issue-2; Mar-Apr, 2024
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Effect of Storage Period on Seed Germination in different Promising Lines of Bambara Groundnut (*Vigna subterranea* (L.) Verdc)

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Received: 21 Feb 2024; Received in revised form: 02 Apr 2024; Accepted: 10 Apr 2024; Available online: 25 Apr 2024

Abstract— *Bambara Groundnut seeds produced in this season often stored for some period of time until the next planting season. Storage period is one of the factors determine the quality of seeds while seeds was stored. This research conducted to determine the effect of storage period on seed germination. Seed viability and seed vigour were observed in 6 promising lines of Bambara Groundnut on 2 different storage period. Experiments was conducted in Agronomy Department, Faculty of Agriculture, Brawijaya University, Malang Indonesia, from May to June 2022. Six promising lines of Bambara groundnut produced from different seasons were stored in cold storage for one year and two years. Each promising line that was stored on respective years was tested with between paper method (BP) according to ISTA. Data collected from the observation of germination, viability characters, vigour characters. Experiments arranged in completely randomized design with combinations of 2 storage period; 1 year, 2 years and 6 promising lines of Bambara Groundnut; CCC 1.6, PWBG 6, PWBG 5.2.1, SS 2.4.2, BBL 1.1, TVSU 86. Results showed that Storage period affect seed viability and vigour. Longer period of storage decreasing some germination characteristics. Seeds stored after 2 years have lower vigour index, final germination percentage and dry weight of shoot and root. Lowest final germination percentage in 2 years storage recorded in PWBG 5.2.1 (70%). Seeds stored on 1 year storage period have higher vigour index (30%-60%), higher germination rate and higher dry weight of shoot and root.*

Keywords— ***Bambara groundnut, germination, promising lines, seed quality, storage period***

I. INTRODUCTION

Bambara groundnut (*Vigna subterranea L.*) is an underutilized legume species originated in Africa and have great potential in terms of their adaptability to climates and high nutritional content [1]. Specifically, Bambara groundnut contain 18-24% protein with high methionine and lysine, 4-12% crude oil content, 51-70% carbohydrates, 3-12% fiber, and 3-5% ash [2]. Bambara groundnut seed have a very hard texture, smooth surface, usually round and vary in size, can reach 1.5 cm in diameter [3]. The yield of Bambara groundnut is strongly influenced by the time of planting. Optimal planting time is in early November to produce high yields and late planting time is around the end of December to January [4]. In Indonesia Bambara groundnut are planted once a year and the seeds are greatly available from March to June [5]. Bambara groundnut takes 4 to 6 months to mature and consumed fresh after boiled or sold directly in the market, while the rest is dried and stored for later [6]. The stored seeds will be planted in the next planting season which can take 6 months to 1 year and during the period of storage deterioration of seeds and damage usually begins to occur.

Storage period is one of the factors determine the quality of seeds while seeds was stored. Seed deterioration occurs mostly due to oxidative processes and aging [7]. Deterioration promotes the breakdown of proteins

(including enzymes), lipids, and then will damage cellular membranes, RNA, and DNA. And all these things adversely affect the cellular integrity and metabolism of seeds and seedlings [8]. When the seed's metabolic system starts to break down, seed ability to germinate decreased and some seeds unable to germinate properly and affect seed viability and vigour. Some seed viability and vigour characters including final germination percentage, germination rate, maximum growth potential of seed and vigour index could greatly decrease after some period of storage.

In the tropical country like Indonesia, high humidity and high temperature can be challenging factor for the storage of orthodox seed storage. Many species of legumes seeds including Bambara groundnut are orthodox seeds. According to the USDA Orthodox seeds are seeds that survive drying and freezing in ex situ conservation and are long-lived seeds. Most seeds remain in good quality for about two to three years but some deteriorate within a year depend on the storage environment. At the time of storage, humidity, temperature, and the proportion of oxygen are the main environmental factors that influence seed deterioration [9]. Storing seeds longer than 1 year becomes a challenge even where seeds are saved, stored, and planted on short annual cycles [10].

Bambara groundnut seeds often stored for one year or more than one year. During period of storage the quality of the seeds in the term of their viability, vigour, and ability to germinate is unknown and there's limited study about effect of period storage on Bambara groundnut. The study of effect of storage period on Bambara groundnut seeds is important to obtain the information about maximum storage period for Bambara groundnut seed and also gaining details about desirable and appropriate temperature and relative humidity for storing Bambara Groundnut seeds.

II. MATERIAL AND METHODS

The experiment was conducted at laboratory of Department of Agronomy, Agriculture Faculty, Brawijaya University, Indonesia from May to June 2022. The study was carried out on six promising lines of Bambara Groundnut. The objective of this study was to evaluate the effect of different storage period on Bambara groundnut germination characters including viability and vigour of the seeds. Seeds used in the experiment obtained from Prof. Dr. Kuswanto M.P. from Department of Agronomy Brawijaya University and produced from different season. All the seeds were stored in the plastic bag under same condition in the cold storage with temperature (24ºC - 26ºC) and relative humidity 40%. Laboratory experiment was conducted with Complete Randomized Design (CRD) with three replicates in multifactorial experiment. The first factor was period of storage (i.e., 1 year and 2 years of storage period) and the second factor was six promising lines of Bambara Groundnut (i.e., CCC 1.6, PWBG 6, PWBG 5.2.1, SS 2.4.2, BBL 1.1, TVSU 86.

Germination test was carried out using rolled paper method in accordance from ISTA recommendations. All seeds and tools were sterilized to prevent fungal contamination before seeds being placed on the surface of the paper. Seeds were soaked in 1% sodium hypochlorite for 5 minutes and rinsed with water afterwards. 10 seeds used for each replication so there's total 30 seeds on each treatment. The paper used to germinate was straw paper substrate. Each paper is cut with size of 26 x 15 cm. Paper substrate was sterilized using oven for 2 hours at 100°C. Three Sheets of straw paper was moistened with distilled water and placed on the prepared plastic sheet. The seeds then planted on straw paper and covered with another 3 sheets of moistened straw paper. Paper then rolled up with plastic paper and placed in the controlled germinator in a standing position. The temperature inside the germination chamber was 24ºC-27ºC. Seeds were observed for 14 days with first count at 7th day and final count at 14th day [5].

Studied germination characters were final germination percentage, seed moisture content, germination rate, vigour index, maximum growth potential of seeds and dry weight root and shoot. Seed moisture content was determined by oven drying method. 5 grams of Bambara groundnut seeds grinded until it forms fine granules and put in the oven for 1 hour at 130ºC.

Final germination percentage (FGP) was observed by calculating the total normal seeds geminate at first count and final count with formula given in [11].

$$FGP\ (\%) = \frac{\text{Germinated Seeds in First Count + Final Count}}{\text{Total Number of Seed Tested}} \times 100\%$$

Germination Rate (GR) is determined by calculating the number of days it takes for the radicle or plumule to appear over a certain period of time calculated by the formula given in [12].

$$GR\ (\%/day) = \frac{N1T1 + N2T2 + \ldots + NxTx}{\text{Total Number of Seed Tested}} \times 100\%$$

Vigour Index (VI) is the ratio between the number of normal germinated seeds on the first count and the total number of seeds planted [13], the total number of normal germinated seed on 7th day (first count) observed on each treatment and calculated by formula given in [14].

$$VI\ (\%) = \frac{\text{Number of Normal Germinated Seeds in First Count}}{\text{Total Number of Seed Tested}} \times 100\%$$

Maximum Growth Potential of Seeds (MGP) determined by calculating the total of both normal and abnormal seedling observed on day 14th (final count) by formula given in [15].

$$MGP\ (\%) = \frac{\text{Number of Normal and Abnormal Seed}}{\text{Total Number of Seed Tested}} \times 100\%$$

Dry weight root and shoot (g) observed on the last day of germination on day 14th (final count) and all normal germinated seeds on the rolled paper were cleaned from the cotyledons and then oven-dried at 80 ^{o}C for 72 hours.

Recorded data analysed using R studio program to perform ANOVA and continued with the Duncan Multiple Range Test (DMRT) test at a significant level of 5%.

III. RESULTS AND DISCUSSION

The results of analysis shows that Bambara groundnut seeds stored for one year and two years indicated that storage period affecting the quality of the seeds both in their viability, vigour and the physical quality of the seeds (Table 1). All the studied characters i.e. seed moisture content, final germination percentage, germination rate, maximum grow potential of seeds, dry weight root and shoot significantly decreased over some period of time.

Seed Moisture Content

All seeds from the promising line that have been stored for 1 year have slightly higher seed moisture content (Table 2) ranged between 12% to 14% with the highest water content recorded is TVSU 86 at 14.38% and the lowest water content is PWBG 6 at 12.94%. Moisture content in seeds stored for 2 years, ranged between 11% to 13% with the highest seed moisture content in seeds stored for 2 years recorded in BBL 1.1 (13.35%). All seeds from six lines of Bambara groundnut both stored after one year and two years has relatively high seed moisture content. High water content of seeds during storage can be caused by the relative humidity around the seeds. Seed is a hygroscopic entity and absorbs moisture from the surrounding, and any change in temperature and relative humidity of the environment, affects moisture contents and quality of the seeds [16].

Table 1. Analysis of Variance from studied parameters

Observation Parameters	Treatment		
	Period of Storage (P)	Promising Line (L)	P x L
Final germination percentage (%)	*	ns	*
Seed moisture content (%)	*	ns	ns
Germination rate (%/day)	**	ns	ns
Vigour index (%)	**	**	**
Maximum growth potential of seeds (%)	**	ns	ns
Dry weight root and shoot (g)	**	**	ns

Note: Note: *: $p < 0.01$; **: $p < 0.05$; ns: not significant

Table 2. Means of studied parameters

Treatments	FGP (%)	SMC (%)	GR (%/day)	VI (%)	MGP (%)	DW (g)
Period of Storage (P):						
1 Year (P1)	94^{a}	13.53^{a}	8.11^{a}	43^{a}	97^{a}	0.31^{a}
2 Years (P2)	87^{a}	12.93^{b}	7.44^{b}	22^{b}	86^{b}	0.18^{b}
Bambara Groundnut						
Lines (G):						

CCC 1.6 (G1)	88^{ab}	13.12^{b}	7.77	25^{b}	88^{ab}	0.25^{ab}
PWBG 6 (G2)	90^{ab}	12.74^{b}	7.94	22^{b}	90^{ab}	0.25^{ab}
PWBG 5.2.1 (G3)	83^{b}	13.19^{ab}	7.69	22^{b}	85^{b}	0.23^{b}
SS 2.4.2 (G4)	90^{ab}	12.93^{b}	7.68	25^{b}	90^{ab}	0.28^{a}
BBL 1.1 (G5)	97^{a}	13.31^{ab}	7.75	55^{b}	97^{a}	0.22^{b}
TVSU 86 (G6)	97^{a}	14.10^{ab}	7.83	48^{a}	97^{a}	0.21^{b}
P X L:						
P1xG1	93	13.57	8.07	40	93	0.32
P1xG2	97	12.94	8.29	30	97	0.33
P1xG3	97	13.16	8.48	40	97	0.28
P1xG4	93	13.89	7.89	47	93	0.35
P1xG5	93	13.26	7.96	60	100	0.28
P1xG6	93	14.38	7.95	43	100	0.28
P2xG1	83	12.66	7.47	10	83	0.18
P2xG2	83	12.53	7.59	13	83	0.17
P2xG3	70	13.23	6.91	3	73	0.18
P2xG4	87	11.96	7.46	3	87	0.21
P2xG5	100	13.35	7.53	50	93	0.16
P2xG6	100	13.83	7.70	53	93	0.15

Note: SMC= Seed moisture content; FGP= Final germination percentage; VI= Vigour Index; MGP= Maximum growth potential of seed; DW= Dry weight of root and shoot; GR= Germination rate.

Other factor affecting moisture content of the seed is improper drying at post-harvest process could result in high moisture content in seeds and later promotes deterioration of the seeds. Study at flowering dogwood seed [17] also showed that seed dried to 14% moisture content germinating poorly after 2 years and failing to germinate after 3 years in storage. SS 2.4.2 stored for 2 years is the only lines which has the lowest moisture content and close to the recommended moisture content for storage (10% or below) as reported by Bonner [18] that orthodox seeds can be stored for relatively long periods if their moisture contents are about 5 to 10%.

Final Germination Percentage

Final germination percentage was observed at first count (7th day) and final count (14th day). After one year period of storage all lines of Bambara groundnut seeds showed higher germination percentage. Higher percentage indicated that the quality of the seed remained high (Table 2). Final germination percentage of six lines are above 90%. PWBG 6 and PWBG 5.2.1 showed highest germination percentage and the rest showed the same result for their germination percentage (93%). During observation all seeds able to germinate to normal seedlings (Figure 1) and no abnormal seedling was recorded. High germination percentage highly associated with high viability of the seeds and recent harvested seeds also tend to have high viability. The viability of seeds is a critical and important factor for seed quality and closely related to resistance to biotic and abiotic stress, germination percentage, and plant performance [19]. For seeds stored after 2 years showed significant decrease of final germination percentage in one of the lines. PWBG 5.2.1 showed lowest final germination percentage (70%) and some seeds grow abnormally (Figure 2). Normal seedlings are seeds that able to germinate with complete structure with all of their essential structures well-developed (Figure 1). Abnormal seeds recorded in PWBG 5.2.1 with some essential structure failed to develop. After 14 days plumule failed to develop and root formed a loop. According to Vujosevic [20] abnormal seedlings are less likely to develop into normal plants. Lower germination and higher abnormality of seedling indicated that deterioration of seeds in Bambara groundnut most likely begin to occur on 2 years period of storage. [21] also stated higher number of abnormal seedlings indicated the deterioration of the seeds.

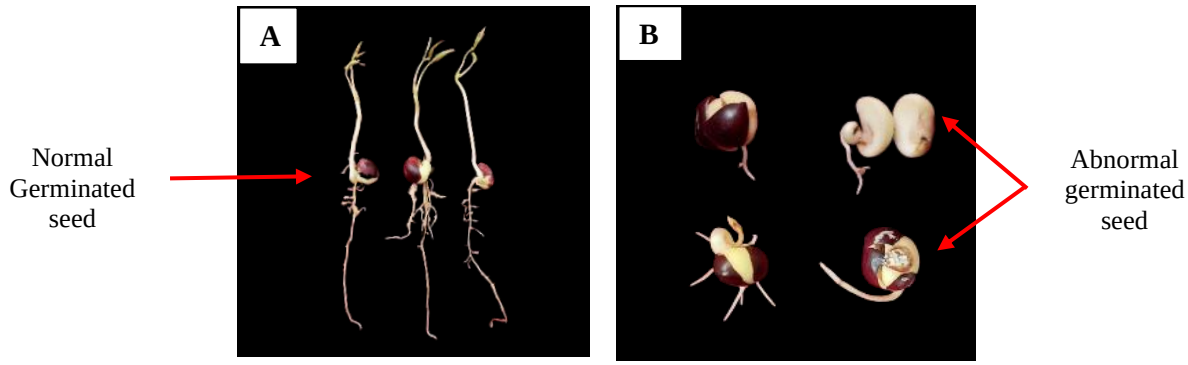

Fig. 1: Seed Germination in Bambara Groundnut: a) Normal germinated seed of Bambara Groundnut with complete essential structure. b) Abnormal growth of Bambara groundnut seed with deformed shape

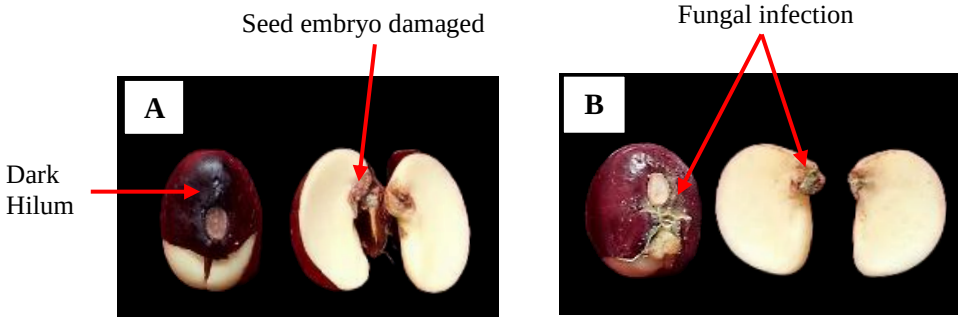

Fig. 2: Dead seeds of Bambara Groundnut: a) Bambara groundnut (PWBG 5.2.1) that unable to germinate stored on 2 years period of storage. b) Damaged embryo of seeds of Bambara Groundnut (PWBG 5.2.1). Stored on 2 years period of storage.

Germination Rate

The results showed significant different in germination rate between Bambara groundnut seeds stored on one year and two years (Table 1). Higher germination rate recorded in all lines of Bambara Groundnut stored for one year with highest germination rate are CCC 1.6 (8.07 %/day), PWBG 6 (8.29 %/day) and PWBG 5.2.1 (8.48 %/day) and SS 2.4.2, BBL 1.1 and TVSU 86 are 7.89 %/day, 7.96 %/day, 7.95 %/day respectively. Faster germination rate on seed stored on one year period of storage indicated that seeds most likely still remained in high viability and vigour and less deteriorated. Siregar [22] also reported, seed with high germination rate associated with decent seed viability.

After 2 years storage, seeds of Bambara groundnut showed slower germination rate with average 7.44 %/day. The rest of the lines showed germination rate above 7 %/day, CCC 1.6 (7.47 %/day), PWBG 6 (7.59 %/day), SS 2.4.2 (7.46 %/day), BBL 1.1 (7.5 %/day) and TVSU 86 (7.70 %/day). Lowest germination rate recorded in PWBG 5.2.1 (6.91 %/day). PWBG 5.2.1 also showed lowest germination percentage and this is the only lines that shows most visible decline of the quality of the seeds compared with all other types. Slower germination rate is one of the symptoms of deteriorated seed because of natural seed aging. Garoma (2017) also reported some parental seed of maize stored on longer duration resulted in delayed onset decreased the germination rate as well as germination index, slow seedling emergence and low weight of seedling traits [23].

Period of storage also show influence to vigour index of the seeds of Bambara groundnut and showed results of highly significant different between 2 period of storage (one year and two years). Vigour index observed by calculating the number of normal seed that able to germinate on the first count (7^{th} day). Vigour index of the seeds stored for 2 years shows most visible decline in most lines of Bambara groundnut. Vigour index ranges from 3% to 50% with lowest vigour index recorded in PWBG 5.2.1 (3%) and SS 2.4.2 (3%) followed by CCC 1.6 (10%) and PWBG 6 (13%). Low vigour can be caused by the storage environment of the seeds. Seed storage is very important to secure good quality seeds for planting materials. Seed longevity, vigour and viability depend on genetic and physiological factors as well as storage conditions. The

most important factors that influence storage are temperature, moisture, seed characteristics, micro-organism geographical location and storage structure [24]. Six promising lines of Bambara groundnut studied stored in plastic bag inside the fridges with temperature 14.4ºC and 40% RH**.** For long term seed storage these conditions could promote rapid seeds deterioration after 3 or 4 years because of the current relative humidity and the temperature. The temperature of fridges is cold, but it also very humid. Factors which influence the longevity of stored seed include harvesting condition, drying process and storing process but the most important is storage conditions which is temperature and relative humidity [25]. As seed stored in suboptimal storage condition and went through ageing phase, seeds physiologically changes including membrane damage and loss of enzymatic activity [26] these physiological changes later contribute to decrease seed vigour

Maximum Growth Potential of Seeds

In this study period of storage does not greatly affecting the maximum growth potential of seeds. Low maximum potential of seeds caused by the high number of seeds that unable to germinate because of severe damaged embryo, hard seed coat and prevent imbibition or fungal infection (Figure 2b) this is linked to post-harvest process and materials used for storing the seeds. Improper post-harvest process and materials to store the seeds may cause seeds damage and further invested with fungi. Taghfir (2018) also reported, the seed container or packaging has an effect on maximum growth potential, germination, growth speed and seed vigour index [27]. Six lines of Bambara groundnut studied, stored in a plastic bag. This material has possibility to create small hole and can be an entry point for pests in seed storage or may promotes fungal infections on the seeds. In order to prevent this, seeds better to be stored in a closed container that is not easily perforated or damaged and it also keep seed humidity low and prevent the seed water content from increasing due to increased humidity [28]. Seed must be stored in moisture-proof containers and stored dry and kept dry to keep the longevity, viability, and vigour of the seeds.

Dry Weight Root and Shoot

Average dry weight of root and shoot from six lines of Bambara groundnut also have different results between two period of storage. Normal germinated seed on one year period of storage recorded 0.307 (gr) and higher than average dry weight of normal germinated seed on two years period of storage (0.176 gr). This result is likely influenced more by seeds vigour and germination rate of the seeds linked with accumulation of dry matter. According to Siregar (2018) Normal seedling biomass indicates abundant nutrient content stored in the plant. High vigour in the seeds contribute to supply energy or the seeds and allow seeds to have enough energy to germinate and supply nutrient stock during the germination process [22], [29], [30].

Physical Change on Seeds

Figure 3a show example of Bambara groundnut seed stored for one year (one year period of storage). The surface structure of the seeds was smooth and the colour of the seeds did not show any change. The colour of seed hilum appears bright and clean. Some of Bambara groundnut seeds stored after 2 years show a little change physically but there were also many damaged seeds found.

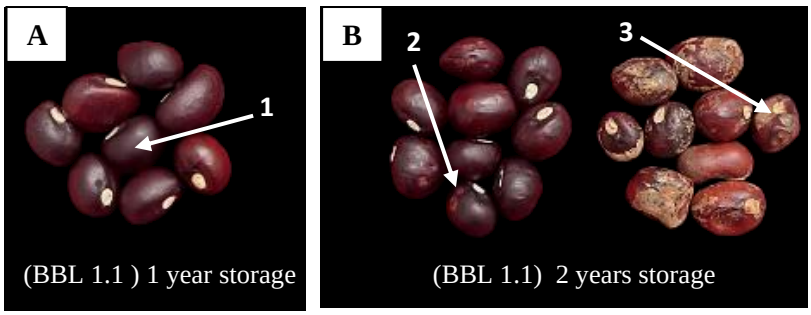

Fig. 3: Bambara Groundnut Seed Stored After 2 Years. Smooth surface of the seed. Discoloration on the surface of the seed (2). Seed Completely damaged (3).

Figure 3b show example of Bambara groundnut seeds on two years period of storage. Seeds shows little change in the surface structure of the seeds, The surface structure of the seed begins to change with the appearance of wrinkles and discoloration in some parts of the seeds but the colour of the hilum was still bright and clean. Figure 3b show example of damaged Bambara groundnut seeds on two years period of storage. Damaged seeds experienced changes in the colour of the seeds to brownish. The seeds become wrinkled and the surface of the seeds becomes rough. The colour of the hilum becomes brown and the seed coat begins to peel. Over period of times Bambara

groundnut seeds went to some natural aging phase and begin to show symptoms of deterioration both physiologically and physically and influence decline in the quality of the seed in terms of their viability, vigour and their ability to germinate. Copeland (2001) also explained that some processes occur in seed deterioration are colour changes in the seeds and the number of died seeds [31], [32].

Pearson Correlation

Pearson correlation between each studied parameter shown in Figure 4. Showed positive correlation among parameters (SMC=seed moisture content, FGP=Final Germination percentage, VI=Vigour Index, MGP=Maximum growth potential, DW=dry weight root and shoot, GR=Germination rate).

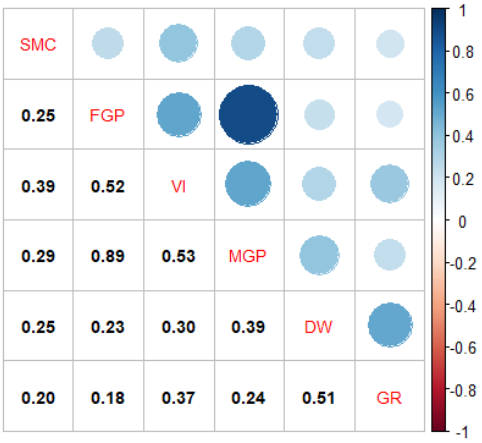

Fig. 4: Pearson Correlation Analysis for Each Parameters

The observation made in this study showed that final germination percentage have positive correlation with seed moisture content (r = 0.25), vigour index (r = 0.52), dry weight root and shoot (r = 0.23), germination rate (r = 0.18) and highly positive correlation with maximum growth potential (r = 0.89). Vigour index shows positive correlation with seed moisture content (r = 0.39), final germination percentage (r = 0.52), maximum growth potential, dry weight root and shoot, (r = 0.30) and germination rate (r = 0.37). Weak correlation found between seed moisture content and germination rate (r = 0.20), final germination percentage with germination rate (r = 0.18), and final germination percentage with dry weight root and shoot (r = 0.23). The further findings in this correlation analysis explain why the decline of germination capacity may have also caused decline in vigour index, germination rate and dry weight root and shoot as there's a positive correlation was found between the parameters. This result describes and emphasized that negative effect of seed deterioration will have an impact on other physiological quality of the seeds.

Germination percentage of seeds highly correlated with maximum growth potential of seed as both parameters observed similar thing but maximum growth potential observed both normal and abnormal seedling. Germination rate have weak correlation with most of the parameters showed that germination rate may not be influenced only with the decline of other parameters but mostly influenced by environmental factors where seed were grown. Temperature and moisture levels are proven to influence the rate of germination [33].

IV. CONCLUSION

Six promising lines of Bambara groundnut stored for 1 year period of storage showed better germination in their ability, viability, vigour and still maintained optimum seed quality. For planting materials seed stored for 1 year suitable for cultivations. This result followed by seeds stored for two years. After two years seeds of six line of Bambara groundnut seed still maintained the quality of the seed but showed some decline in some germination characters and greatly affecting their vigour and germination rate. After 2 years stored, seeds began to show symptoms of deterioration both in their germination ability and also physical deterioration symptoms also occurred. This decline and deterioration process may cause by high moisture content of seeds when seeds stored and later accelerate because of the environment condition of the storage chamber (low temperature but high relative humidity).

In conclusion Bambara groundnut seeds can be stored for 2 years while still maintaining their quality with some seeds showed symptoms of deterioration. Maximum storage period for Bambara groundnut may reached 3 years or more if the seeds stored in lower temperature and lower relative humidity so it can delay or prevent accelerate severe seed deterioration.

ACKNOWLEDGMENT

This research was funded through Hibah Professor program and author would like to express our gratitude to supervisors and co-supervisors for the support and fund assistance during this research.

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